

Amendments to the Specification:

Please replace lines 5 and 6 on page 5 with the following amended lines 5 and 6:

“Figure 11 graphically shows an increase in the percent apoptotic cells with increasing doses of the inhibitor XX5 ((4-(3-Butoxy-4-methoxybenzyl)-2-imidazolidinone)(Ro-1724)).”

Please replace the paragraph bridging pages 6 and 7 with the following amended paragraph:

B. Phosphodiesterases As A Target For Therapy Of CLL

Cyclic AMP is catabolized within cells to 5'-AMP by 3':5' cAMP phosphodiesterases (PDE), a diverse group of enzymes encompassing 15 gene products and 7 classes of enzymes which have proven to be the target of successful pharmaceutical agents for neurologic, cardiovascular and inflammatory disorders. Despite this large array of cyclic nucleotide PDEs, only a subset of these enzymes have been reported in human lymphoid cells. Among them, the most commonly reported enzymes in human T cells are types 1, 3 and 4. Calcium-calmodulin dependent type 1 PDE activity has been detected in phytohemagglutinin-stimulated but not resting peripheral blood lymphocytes. One isoform from this family, PDE1B1, was recently detected in acute lymphocytic leukemia cells; inhibition of this enzyme was reported to induce apoptosis. PDE1 enzymes, which can catalyze the degradation of both cAMP and cGMP, are specifically inhibited by vinpocetine (IC₅₀ = 21 mMol/L). Two groups have reported both type 3 and type 4 PDE in human T lymphocytes; lectin-mediated proliferation was completely suppressed only by treating cells with specific inhibitors of both classes of enzymes. While four human PDE4 genes have been cloned, only three of the isoforms (PDE4A, B and D) have been identified in lymphocytes. Type 4 enzymes are specifically inhibited by rolipram [4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone] (IC₅₀ = 1 mMol/L) and the structurally related compound XX5 ((4-(3-Butoxy-4-methoxybenzyl)-2-imidazolidinone)(Ro-1724))(IC₅₀ = 2 mMol/L). U. Schwabe *et al.*, “4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone (ZK 62711): a potent inhibitor of adenosine cyclic 3',5'-monophosphate phosphodiesterases in homogenates and tissue slices from rat brain,” *Molecular Pharmacology* 12:900 (1976). H. Sheppard *et al.*, “Structure-activity relationships for

inhibitors of phosphodiesterase from erythrocytes and other tissues," *Adv Cyclic Nucl Res* 1:103 (1972).

Please replace the paragraph bridging pages 18 and 19 with the following amended paragraph:

As a more quantitative analysis of CLL apoptosis, we utilized a flow cytometry method in which apoptotic cells are distinguished both by their reduced size (FSC) and their increased uptake of the lipophilic UV fluorescent dye Hoechst 33342 (FL-4) when the intact, heterogeneous cell population is incubated with a low concentration of the dye (.25 ug/mL) for 10 minutes at 37°C. Specifically, cells were cultured for 72 hours in media (1), 1 uMol/L rolipram (2), 40 uMol/L forskolin (3) or a combination of the two drugs (Figure 6). The abscissa reflects forward light scatter and the ordinate Hoechst 33342 fluorescence. Apoptotic cells are characterized by reduced forward light scatter and increased Hoechst 33342 fluorescence. Previous reports of cAMP induced lymphoid apoptosis have noted that this form of programmed cell death may take 48 to 72 hours to develop maximally. Using the Hoechst 33342 assay in a time course experiment, we found that the combination of 10 uMol/L rolipram and 40 uMol/L forskolin induced significant CLL apoptosis which plateaued 48 to 72 hours after the addition of these drugs (Figure 7, left panel). CLL cells were cultured for 72 hours in one mL of media with the indicated concentration of rolipram with (black bars) or without (stippled bars) the addition of 40 uMol/L forskolin. Using the 72 hour culture period, we found a dose dependent increase CLL cell apoptosis when leukemic cells were incubated with rolipram (Figure 7, right panel). Treatment of CLL cells with forskolin alone induced moderate apoptosis, but combination of forskolin with even low doses of rolipram resulted in a supra-additive effect on induction of CLL apoptosis (Figure 7, right panel). Similar results were obtained with a structurally distinct PDE4 inhibitor, XX5 ((4-(3-Butoxy-4-methoxybenzyl)-2-imidazolidinone)(Ro-1724)), or when isoproterenol or prostaglandin E2 were utilized to activate CLL adenylate cyclase rather than forskolin (data not shown).

Please replace the paragraph bridging pages 22 and 23 (EXAMPLE 7) with the following amended paragraph:

EXAMPLE 7

It is not intended that the present invention be limited to only one particular inhibitor. The present invention contemplates the treatment of patients with chronic lymphocytic leukemia (CLL) with a variety of inhibitors that specifically inhibit Type 4 cyclic adenosine monophosphate phosphodiesterase. For example, Figure 11 graphically shows an increase in the percent apoptotic cells with increasing doses of the inhibitor XX5 ((4-(3-Butoxy-4-methoxybenzyl)-2-imidazolidinone)(Ro-1724)). One million purified CLL cells were cultured for three days in media (control), 40 uM forskolin (F) and/or the indicated concentrations of the PDE4 inhibitor XX5 ((4-(3-Butoxy-4-methoxybenzyl)-2-imidazolidinone)(Ro-1724)). Cells were then harvested and analyzed for apoptosis using the Hoechst 33342 FACS assay. SEM of triplicate cultures is shown.